Rapid lytic activity of bacteriophages against planktonic and biofilm methicillin-resistant *Staphylococcus aureus* (MRSA) by microcalorimetry

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INTRODUCTION

- Biofilms on implants are causing persistent and difficult to cure infections, which remain an unresolved challenge. Alternative treatment strategies needs to be explored.
- Bacteriophages (Fig. 1) are promising for eradication of biofilm because of high selectivity and rapid bactericidal activity, including on multi-resistant bacteria.
- Their activity against bacterial biofilms have not yet been characterized. In this study we investigated the activity of lytic bacteriophages against *Staphylococcus aureus* in planktonic and biofilm form using isothermal microcalorimetry, measuring growth-related heat production.

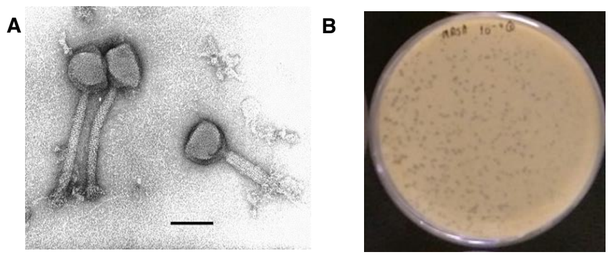

Figure 1. (A) Electron micrograph of Sb-1 phage (Kvachadze et al.). (B) Plaques formed by Sb-1 phage

MATERIAL & METHODS

- *S. aureus* specific phage (Sb-1) and Pyo-bacteriophage cocktail (containing different strain-specific bacteriophages) were tested against MRSA (ATCC 43300).
- Microcalorimetry was performed using an isothermal calorimeter at 37° C in sealed glass ampoules containing brain heart infusion (BHI).
- MRSA (5 x 10^5 cfu/ml) was exposed to different bacteriophages titre (MOI = multiplicity of infection, given by the ration of N° of bacteria / N° of phages).
- MRSA biofilm was formed on porous glass beads and incubated for 24 h at 37° C in BHI, washed and exposed to different concentration of bacteriophages. Heat flow (μW) and total heat (J) were measured for 24 h and 48 h, respectively.

RESULTS

The growth of planktonic MRSA was inhibited in a concentration-dependent manner with MOI ranging from 0.001 to 10. The heat production was completely abolished at MOI 10. Against biofilm MRSA, both phages inhibit >90% of biofilm growth with the highest titre. Pyo-phage showed a better activity than Sb-1 at a lower titre load.

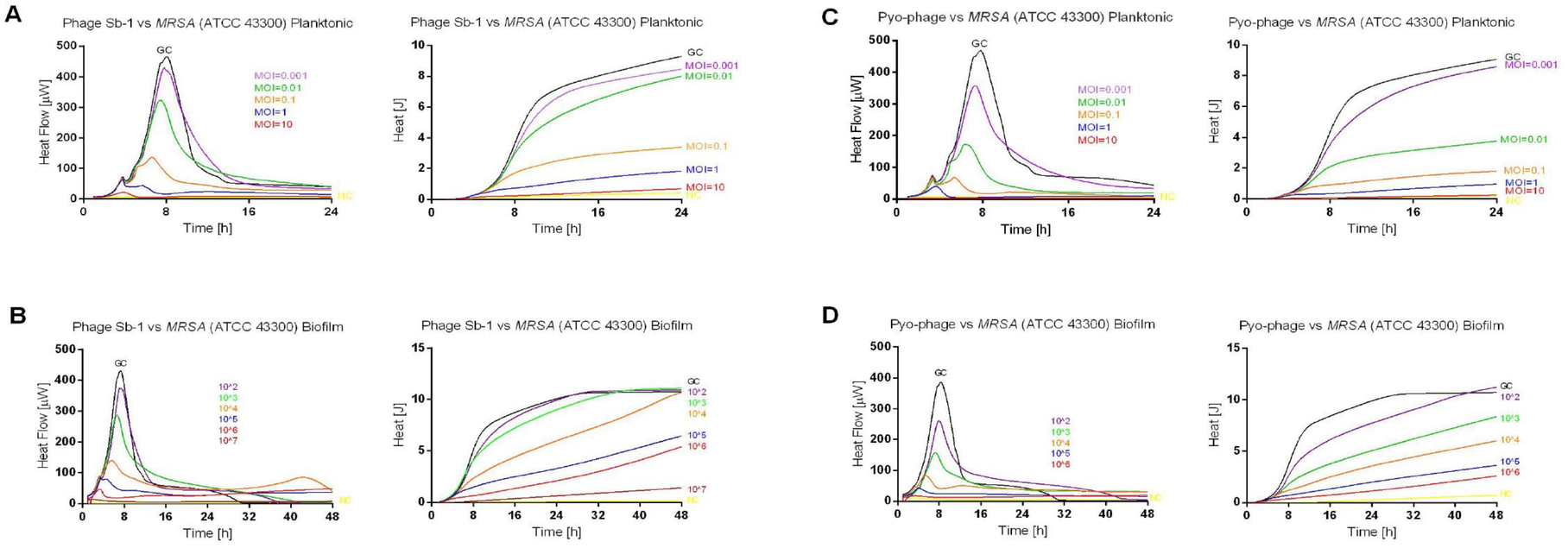

Figure 2. Heat flow (μW) and the total heat (J) generated by MRSA after incubation with phages: (A) Heat produced by planktonic bacteria (10^5 cfu/ml) after exposure to Sb-1 [MOI 0.001-10]. (B) Heat produced by biofilm bacteria in presence of Sb-1 [10^2-10^7 pfu/ml]. (C) Heat produced by planktonic bacteria in presence of Pyo-phage [MOI 0.001-10]. (D) Heat produced by biofilm bacteria in presence of Pyo-phage [10^2-10^7 pfu/ml]. MOI = multiplicity of infection; GC = growth control; NC = negative control.

CONCLUSIONS

- Both bacteriophages (Sb-1 and Pyo-phage cocktail) rapidly inhibited growth of planktonic and biofilm MRSA in a concentration-dependent manner.
- A complete inhibition of planktonic MRSA was achieved with an MOI = 10 and both phages inhibit >90% of MRSA biofilm growth with the highest phage titre.
- Phages showed to be a promising tool for local and systemic treatment against planktonic and biofilm bacteria.
- The potential synergistic activity of bacteriophages combined with antibiotics should be explored.